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Genetic characterization of multi resistant *Listeria monocytogenes* isolated from rabbits

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Research

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Abstract

Rabbit's breading becomes one of the most substantial sources of revenue world-wide. Commonly, rabbits are susceptible to some serious bacterial illnesses such as listeriosis which is an etiology of grave economic losses. Nonetheless, Listeria has developed an impressively various array of genetic components for gaining fostered tolerance to not only metals, but also to antibiotics. The genus *Listeria* currently includes seventeen recognized species exist throughout the environment. The current study firstly surveyed the prevalence of Listeria spp., primarily *Listeria monocytogenes* in rabbit meat and offal. Secondly, determine the genetic characterization of *Listeria monocytogenes* resistance to arsenic metals in addition to the assessment of the antibiogram of the identified *Listeria monocytogenes*. Out of two hundred rabbit samples, three *Listeria* spp. namely, *L. monocytogenes*, *L. ivanovii* and *L. grayi* were biochemically identified with a percentage of 7%, 1% and 0.5%, respectively. The most predominant species is *L. monocytogenes*. Antibiotic sensitivity test showed that *L. monocytogenes* isolates revealed a resistance rate of (100%) to Clindamycin, Colistin sulphate, Sulphamethoxazole- trimethoprim. The genetic determination of *L. monocytogenes* isolates showed that (13 /14) of isolates harbored *mef*A (92.9%), while 11/14 isolates (78.5%) expressed *amp*C, otherwise 10/14 (71.4%) isolates harbored *aad*6 and only 5 (35.7%) isolates expressed *ars*B. In conclusion, this study demonstrated resistant *L. monocytogenes* isolates which found to be harbored antimicrobial resistance components in rabbits which could threaten safety and institute serious health consequences if consumed. Application of good sanitation programs as well as successful preventive and control measures in rabbit farms should be recommended.

***Keywords**: Multidrug resistance, arsenic, Listeria and rabbit.*

Introduction

Rabbit's industry has been evolved in Middle-Eastern nations comprising Egypt due to numerous merits involving high fertility rate, good feed conversion ratio, rapid growth rate, short production cycle, as well as big productive ability **Cullere and Dalle Zotte (2018**). Additionally, rabbit's meat has acquired popularity as the healthiest meat source because of containing high level of protein with low levels of sodium, fat and cholesterol besides ease digestibility, **Wang *et al.* (2019**). However, rabbit meat is susceptible to numerous serious bacterial infections during slaughter, processing, evisceration and transportation that affect human health and productivity such as

listeriosis **Darwish *et al.* (2018).** Due to the adverse effect of listeriosis on rabbits' productivity as well as its food-born zoonosis, the organism represents a great public health concern **Johansson and Freitag (2019**).

The genus Listeria involves seventeen recognized species: the most significant are *L. monocytogenes, L. ivanovii, L. grayi,* and *L. innocua.* The other species are *L. aquatic, L. booriae, L. fleischmannii, L. floridensis, L. grandensis L. marthii, L. newyorkensis, L. riparia, L. rocourtiae, L. seeligeri, L. welshimeri,* and *L. weihenstephanensis* **Orsi and Wiedmann (2016**). Rabbits looked as a natural host for *L. monocytogenes* and the meat of affected animals constitutes a substantial source of human food-borne infection **Zhao *et al.* (2020**). Listeriosis infection in rabbits with *L. monocytogenes* causes, abortion, infertility and high mortality with grave losses in rabbits **Abd El-Ghaffar and Abd-Elgwad (1997**). Despite *L. monocytogenes* may exhibit low incidence rate, it often linked with high mortalities **Radoshevich and Cossart (2018**). The pathogen was isolated from affected farmed rabbits in Egypt with frequency of 21.8% **Ibrahim and Ibrahim (2016**).

L.monocytogenes is a ubiquitous saprophytic bacterium and a facultative intracellular pathogen, It can also be recovered from the soil, vegetation and water **Gray *et al.* (2006**). Animal infection with *L. monocytogenes* is often linked to dietary origin as, stored forage **OIE (2018)**. This transmission is assisted by the pathogen's capability to grow and form biofilm at cold temperatures even in refrigerator. Thereby, the risk of cross-contamination through food processing and storage was elevated **Colagiorgi *et al.* (2017)**. Therefore, in Egypt, continuous observing for the incidence rates of *L. monocytogenes* in retailed meat and viscera is of subject of concern for consumer's safety as well as food hygiene.

Nowadays, antimicrobials are broadly used in the veterinary field. The random unrestricted use of antimicrobials or their use in subtherapeutic dosing can result in the emergence of antimicrobial resistant strains, which is a serious concern not only in animals but also in human health **Beninati *et al.* (2015)**. Recently, the elevation of antimicrobial resistance of *L. monocytogenes* reports has been noticed. This issue constitutes a dangerous threat to the therapeutic mitigation of listeriosis infection.

On the other hand, heavy metals such as iron, copper and zinc are cofactors for numerous cellular processes and important for cellular survival **Argüello *et al.* (2013**). Other heavy metals such as cadmium and arsenic, however, are innately toxic, hindering cellular processes and inducing cell death over a certain threshold. These toxic metals either can be exist in the environment naturally or may be as the results of human factor pollution. Environmental dissemination of these toxic metals tends to be heterogeneous, and only in certain ecosystems they are typically encountered at high levels **Nunes *et al.* (2016**). Nonetheless, toxic heavy metal resistance genes are broadly found in *Listeria* and other Gram +ve bacteria. These genes are loaded on chromosomes and/or on mobile genetic elements (MGEs) such as plasmids and transposons or conjugative and integrative elements (ICEs) **Argudín *et al.* (2019**). These determinants can thus be widely distributed and highly conserved that they can disseminate via horizontal gene transfer (HGT).

In light of the preceding facts, the target of this study was to assess the phenotypic detection of Listeria spp., specifically *L. monocytogenes*, then investigate the incidence of *Listeria* spp. in rabbits' colonies in Egypt. Also, detection of the antibiogram of the detected isolates, and finally genetic determination of antibiotic resistance genes as well as arsenic metals resistance determinants in *L. monocytogenes* obtained from rabbits.

Materials and Methods

1- Sample collection and identification

From March to September 2022 a total of 200 sample from apparently healthy and clinically diseased rabbits (different ages) were collected from farms located in Giza and Dakahlia governorates. The collected rabbit showed anorexia, lethargy, vaginal purulent discharge, intrauterine fetal death, conjunctivitis, lacrimation, depression, ruffling fur, retention of urine, nervous signs in the form of lateral deviation of the head and convulsions, diarrhea soiled hind quarters persuaded by emaciation then death. The post-mortem examination of the

diseased rabbits reveals hepatomegaly. Additionally, hemorrhagic lesions were noticed in the uterus, with thickening of the uterine wall.
Samples were aseptically collected from brain, liver, aborted fetus and vaginal swabs (50 from each animal), then labeled and transported directly in an ice box to be examined in Reference Laboratory for Veterinary Quality control on Poultry production (RLQP) (Dokki, Giza).

2- Isolation and identification of Listeria.

Listeria was isolated from the examined samples according to **ISO 11290-(2017).** Samples were diluted one tenth using Half-Fraser broth (Oxoid) subsequently; all samples have undergone homogenization in a Stomacher® (West Sussex, UK) for one minute. The broth was incubated at 30°C for twenty four hours. The vaginal swabs were pre-moistened using ten millimeters of buffered peptone water then vortexed and diluted one tenth in Half Fraser broth and stored at 37°C for twenty four hours. Then, one millimeter from the Half-Fraser broth was transferred into nine millimeters of Fraser broth (Oxoid) and kept at 37°C for 2 days.
A loopful of the incubated Fraser broth was cultured onto PALCAM agar plates, and incubated at 37°C for forty eight hours and examined after 24-30 hours. After that, any positive colonies were streaked onto ALOA agar and Oxford agar plates, which were also incubated at 37±1°C for 48 hours and examined after 24±3 hours. Suspected Listeria colonies were selected and streaked onto Tryptic Soy agar supplemented with 0.6% yeast extract (TSA-YA) and incubated at 35°C for forty eight hours. Thereafter, the isolates were morphologically defined by Gram' stain in accordance with **Quinn *et al.* (2011).**

3- Phenotypic and biochemical identification of Listeria.

Suspected colonies for Listeria spp. have exhibited certain morphological merits; dew drop -like, dark brown or black with brown hallow colonies, 1-2 mm in diameter. For biochemical identification, a series of biochemical tests comprising oxidase test, catalase test, urease test, nitrate reduction test, Voges-Proskauer test (VP), methyl red (MR), esculin test, carbohydrate fermentation, gelatin hydrolysis, and motility tests were carried out as described by **Markey *et al.* (2013).** Also, acid production from (Xylose, Rhamnose and Mannitol), hemolysis test and CAMP test were applied to identify the species of a Listeria isolate this test was applied using standard strain of *Staphylococcus aureus* (ATCC) (25923) **Molla *et al.* (2004)**.

4- Antibiotic sensitivity test of *L. monocytogenes*

The recovered bacterial isolates were tested in vitro for their antibiotic sensitivity test using disc diffusion method the following antimicrobial discs: Cefotaxime (CTX) 30 µg, Doxycycline (DO) 30 µg, Enerofloxacin (ENR) 5 µg, Colistin sulphate (CT) 25µg, Clindamycin (DA) 2 µg, Chloramphinicol (C) 30 µg, Erythromycin (E) 15 µg, Ampicillin (AMP) 10µg, Streptomycin (S) 10µg and Sulphamethoxazole - trimethoprim (COT) 1.25/23.75 µg. according to **Bauer *et al.* (1966)**. The degree of sensitivity was interpreted following European Committee on Antimicrobial Susceptibility Testing **EUCAST (2017**) and **CLSI (2020)**. The examined isolates were evaluated as resistant (R), intermediate (I) and susceptible (S). Multiple Antibiotic Resistance (MAR) index for each isolate was evaluated following the formula provided by **Singh *et al.* (2010**); (MAR) index = a/b

a: Number of resistant isolates, (the isolates that were classified as intermediate were looked susceptible for MAR index)

b: Total number of tested antibiotics.

5- Molecular identification of *L. monocytogenes* and detection of resistance genes.

5-1. DNA extraction.

QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used for DNA extraction from samples with modulations, following the manufacturer's recommendations. In detailed, two hundred microliters of the sample suspension was incubated with twenty microliters of proteinase K and two hundred microliters of lysis buffer at 56^{O}C for ten minutes. After incubation, add two hundred microliters of 100% ethanol to the lysate. Then the sample was washed and centrifuged according to the manufacturer's instructions. Nucleic acid was eluted with one hundred microliters of elution buffer stocked in the kit. Oligonucleotide primers used were purchased from Metabion (Germany) and mentioned in **Table (1).**

5-2. PCR amplification technique.
Primers were utilized in a twenty five microliters reaction. The reaction comprised 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), one microliter of each primer of twenty pmol concentrations, 5.5 µl of water, and five microliters of DNA template. The reaction was conducted in an Applied biosystem 2720 thermal cycler.

5-3. Analysis of the PCR Products.
The products of PCR were electrophoresed on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, twenty microliters of the products were loaded in each gel slot. Two ladders; Generuler 100 bp (Fermentas, Thermo, Germany) and Gelpilot 100 bp plus (Qiagen, Germany, GmbH) were used to define the fragment sizes. The gel was photographed via gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

6- Evaluation of the antibiotic resistance genotyping patterns of *L. monocytogenes* isolates.
Isolates that carried multiple resistance genes were profiled, and the multiple antibiotic resistance genotype (MARG) patterns were mentioned including the *mef*A, the *amp*C, and *aad*6

Results

1- Detection and prevalence of *L. monocytogenes*.
A total of 17/200 (8.5%) Listeria spp isolates were isolated from rabbit brain, liver, aborted fetus and vaginal swabs with the isolation rates of 16%, 8%, 6%,4 %, respectively (**Table, 2)**. On PALCAM agar, the colonies grew well and revealed gray-green color with a black depressed center and surrounded by black halo. On the other side, the isolates formed black colonies with dimpled centers on Oxford agar, while they displayed green-blue colonies surrounded by an opaque halo on ALOA agar. The organisms were identified as Gram +ve bacilli or coccobacilli, exposing motility at room temperature on semisolid TSA- YA agar, characterized by umbrella growth and ”templing" motility behavior.
Biochemical identification of the obtained *Listeria* spp. displayed three *Listeria* spp. namely; *L. monocytogenes*, *L. ivanovii* and *L. grayi* with a percentage of 7%, 1% and 0.5% %, respectively. The most predominant spp is *L. monocytogenes* which was detected in 14 samples (14/200). Virulence testing demonstrated that 100% of *L. monocytogenes* isolates were positive for CAMP test, and exhibiting a zone of β-hemolysis at the interface with *S. aureus* strains.

2- Results of the antibiotic sensitivity test
Results of the antibiotic susceptibility test in **Table (3)** showed that all the tested *L. monocytogenes* isolates exhibited resistance against Colistin sulphate, Clindamycin, and Sulphamethoxazole- trimethoprim (100%) followed by Cefotaxime, Ampicillin Erythromycin, Doxycycline, Streptomycin, Chloramphinicol, and Enerofloxacin, with a percentage of (92.85%), (92.85%), (92.85%), (85.71%), (85.71%), (64.28%) and (35.71%), respectively. Interestingly, all the *L. monocytogenes* isolates were MAR; exposing resistance to more than 2 classes of antibiotics, and revealing 7 resistance patterns. The MAR index of the strains ranged from 0.6 to 1 as described in **(Table, 4).**

3- Genotypic detection of some resistance genes of *L. monocytogenes* isolates with PCR:
(**Fig. 1**) showed that Fourteen out of 17 isolates were confirmed as *L. monocytogenes* isolate through detection of 16SrRNA gene. (**Fig. 2**) showed that (13 /14) of obtained isolates harbored macrolides resistance gene (*mef*A) (92.9%), while 11/14 isolates (78.5%) expressed β-lactamases resistance gene (*amp*C) **(Fig. 3)**, moreover 10/14 (71.4%) isolates harbored aminoglycosides resistance gene (*aad*6) **(Fig. 4)** and only 5 (35.7%) isolates expressed arsenic resistance gene (*asr*B) **(Fig. 5)**, **(Table, 5).**

Discussion

Listeria spp. is opportunistic intracellular bacterium that has the ability to survive under extreme pH, temperature and osmolarity **Pasquali, *et al.* (2018**). Listeriosis is considered a disease of clinical and economic importance for the rabbit industry. Direct contact with contaminated materials, such as aborted fetuses, may be a potential route for *L. monocytogenes* infection in humans. **Abd El-Ghany (2023).** Contamination of rabbit meat with foodborne

pathogens has pointed the bad hygienic measures embraced during processing of such a substantial meat source **Darwish *et al.* (2018)**.

The data of our study revealed that *Listeria* spp. was isolated from rabbit brain, liver, vaginal swabs and aborted fetus at 16%, 8%, 4%,6%, respectively**.** The prevalence of *Listeria* spp. from rabbit in the current study was 8.5 %. This level is near to the isolation rate (11%) of Listeria spp. from rabbit meat products retailed in Italy **De Cesare *et al.* (2017).**

Biochemical identification of the obtained Listeria spp. displayed the isolation of three Listeria spp. termed, *L. monocytogenes*, *L. ivanovii* and *L. grayi*. The most predominant spp. *L. monocytogenes* which was detected in 14 samples (7%). Prevalence ratio of *L. monocytogenes* in this study, was (7%) closely similar to percentage (11.11) in rabbits which was reported by **Abd El Tawab *et al.* (2018)**. While incidences lower than (20% and 21.73%) were recovered from rabbit farms by **Hatab and Abd El-Ltif, (2006) and Morsi *et al.* (2006**), respectively. *L. monocytogenes* still occurs at a low prevalence **Gómez *et al.* (2014)**. Despite the low incidence of listeriosis its linkage with high mortality rates represents a grave health concern **(Datton *et al.* 2004).** The variations in the prevalence rate may be contributed to variance in sampling procedure, locality, the season of sample collection and variation in the method of isolation.

In the current study the antibiogram of *L. monocytogenes* isolates (14) were tested against 10 antibiotics using the disk diffusion method **(Table, 3).** The results revealed antimicrobial resistance to colistin sulphate (100%), clindamycin (100%), sulphamethoxazole- trimethoprim (100%). cefotaxime (92.85%), erythromycin (92.85%), ampicillin (92.85%), doxycycline (85.71%), streptomycin (85.71%), chloramphinicol (64.28%) and enerofloxacin (35.71%), and Most of isolates (92.85%) were resistant to β-lactams antibiotic family (Ampicillin and cefotaxime). These results have a great importance because β-lactams consider the drugs of choice for human listeriosis **Olaimat *et al.* (2018)**. Concerning to high resistance to ampicillin which is the first-choice treatment for listeriosis so this represents a major public health concern) **Koopmans *et al.* (2023).**

Nearly parallel results were determined by **Abd El Tawab *et al.* (2018)** for Colistin (90%) **Rodrigues *et al.* (2024)** for Sulfamethoxazole-trimethoprim (79%). On contrast, lower results were obtained by **Abd El Tawab *et al.* (2018)** for Cefotaxim (40%), Doxycycline (40%) and Erythromycin (20%). Also, lower results for Ampicillin (44.98%), Sulfamethoxazole-trimethoprim (37.54%), Erythromycin (30.11%) were obtained by **Rippa *et al.* (2024)**. Generally, the data of the current study differ from those mentioned by **Caruso *et al.* (2020)** who reported a high sensitivity of isolates to Ampicillin, Erythromycin and Sulfamethoxazole-trimethoprim (100%) for each.

In the current study 100% of the *L. monocytogenes* isolates were MAR and the MAR index of these isolates ranged from 0.6 to 1 **(Table, 4).** Lower results were obtained by **Elbar *et al.* (2020)** who stated that 37.5% (6/16) of the L. monocytogenes strains were MAR and the MAR index ranged from 0.1 to 0.6. Pathogens with a MAR index value less than 0.2 would come from a lower-hazard origin, but those with value increasing 0.2 would typically derived from a higher-risk origin and reflect improper use of these antibiotics.

The high resistance of *L. monocytogenes* may be attributed to the selective pressure imposed the overuse use of various antimicrobial agents which resulted acquiring target gene mutations in members of this genus and resistance-encoding genes on mobile genetic elements **Morvan *et al.* (2010)**. Transfer of resistance determinants between Listeria species and other bacteria, such as *Enterococcus* and *Streptococcus* by self-transferable plasmids has also been determined **Lungu *et al.* (2011)**.

The use of 16S rRNA gene of *L. monocytogenes* is assumed as a perfect signature for a bacterial species and had become the technique of choice for defining and discriminating microorganisms particularly when no other easily specified nucleic acid sequence determines the required target **Czajka *et al.* (1993**). In the present study, PCR displayed clear bands at 1200 bp as presented in **Fig. (1**) referring that all tested isolates (100%) harbored *16SrRNA* gene asserting the bacteriological identification of *L.*

monocytogenes and discriminating it from other *Listeria* species. This data have agreed with **Soad *et al.* (2016**) who reported that all eight isolates exhibited amplification of 16SrRNA.

L. monocytogenes strains were assessed by PCR for the resistance genes, *mef* (A) gene that encoded for the expression of macrolide, *aad*A gene that encoded for the expression aminoglycoside, *amp*C gene that encoding β-lactamase resistance (ESBL). Resistance to 14- and 15-membered-ring macrolides is described in Gram-positive bacteria, and be mediated by drug efflux pumps belonging to the ATP-binding cassette transporter to the major facilitator superfamily *mef* (A) gene **Leclercq (2002**).

In the current study as shown in **Table (4)** and **Table (5)** the data revealed that 13/14 (92.85%) of the examined *L. monocytogenes* isolates harbored *mef*(A) gene which appeared phenotypically resistant with the same percentage. **Wi´sniewsk *et al.* (2022)** detected *mef*(A) with a percentage 25.5% but **Escolar *et al.* (2017) and Granier *et al.* (2011)** failed to detect *mef* (A). Meanwhile, *amp*C gene was detected in 11/14 (78.6%) which appeared phenotypically resistant in 13/14 (92.85%) while, **Duma *et al.* (2024)** detected that 58% of the examined isolates harbored *amp*C resistance gene while, **Davis and Jackson (2009)** failed to detect any resistance gene related to β-lactams and aminoglycosides. Concerning to *aad*6 gene it was detected in 10/14 (71.4%) of the examined isolates that was appeared phenotypically resistant in 12/14 (85.71%) while **Morvan *et al.* (2010)** failed to detect *aad*6 gene while the isolates were phenotypically resistant and attributed that this resistance could be due to ribosomal mutations.

Arsenic resistance has been foremost linked with serotype 4b, which is over-shown among clinical strains in comparison to those from foods and food processing environments **Lee *et al.* (2017**). Non-pathogenic *Listeria* species seem to lack arsenic resistance, **Glaser *et al.* (2001**). These findings assume that arsenic resistance is mainly encountered in *L. monocytogenes*, particularly in serotype 4b. In the current study only 5 (35.7%) isolates expressed *ars*B. Also, **Parra-Flores *et al.* (2022)** reported that the *ars*BC, was detected which conferred resistance to stress and disinfectants. Further studies revealed arsenic-resistant isolates within serotype 4b was most frequently encountered among clones associated with outbreaks **Lee *et al.* (2014); lee *et al.* (2013) and Lee *et al.* (2017).**

Conclusion

It could be concluded that, multidrug resistant strains of *L. monocytogenes* are serious pathogens that have clinical and productive significance for rabbits industry. Improper use of antimicrobials poses a significant threat to public health so It's crucial to identify safe alternatives to antibiotics for raising rabbits in intensive farming systems in Egypt. Also, the mechanism and role of other virulence determinants of *L. monocytogenes* isolates in rabbit should be in vivo and in vitro investigated.

Table (1). Primer's sequence used for detection *L. monocytogenes* and some resistance genes.

Target gene	Primers sequences	Amplified segment (bp)	Reference
16S rRNA	F-GGA CCG GGGCTA ATA CCG AAT GAT AA	1200	**Kumar *et al.* (2015)**
	R-TTC ATG TAGGCG AGT TGC AGC CTA		
*Mef*A	F-AGTATCATTAATCACTAGTGC	345	**Morvan *et al.* (2010)**
	R-TTCTTCTGGTACTAAAAGTGG		
*amp*C	F- TTCTATCAAMACTGGCARCC	550	**Srinivasan *et al.* (2005)**
	R- CCYTTTTATGTACCCAYGA		
*Ars*B	F- GTSAARCCSTTYTCGATGGC	226	**Mustafa *et al.* (2021)**
	R- GCRAASGCSAHSAYCATGAT		
*aad*6	F- AGAAGATGTAATAATATAG	978	**Morvan *et al.* (2010)**
	R- CTGTAATCACTGTTCCCGCCT		

Table (2). Incidence rates of Listeria spp. obtained from the addressed rabbit samples.

Samples	Number	Positive	%	Prevalence of the biochemically identified *Listeria* spp. isolated from the tested rabbit samples					
				L.monocytogenes	%	*L.ivanovii*	%	*L. grayi*	%
Brain	50	8	16	6	12*	1*	5.8*	1*	2*
Liver	50	4	8	4	8*	-	-	-	-
Vaginal swabs	50	2	4	2	4*	-	-	-	-
aborted fetus	50	3	6	2	4*	-	-	1	2*
Total	200	17	8.5	14	7**	1**	0.5**	2**	1**

* Percentage according to number of examined samples for each organ
** Percentage according to number of total numbers of examined samples.

Table (3). Anti-microbial sensitivity test for isolated *L. monocytogenes* by disc diffusion

Antimicrobial disc	Disk concentration	Number of sensitive isolates (%) *	Number of interme-diate isolates (%) *	Number of resistant isolates (%) *
Cefotaxim	30 µg	1 (7.14%)	0 (0%)	13 (92.85%)
Doxycycline	30µg	2(14.28%)	0(0%)	12(85.71%)
Enerofloxacin	5 µg	8 (57.14%)	1(7.14%)	5(35.71%)
Colistin sulphate	25µg	0 (0%)	0 (0%)	14 (100%)
Clindamycin	15 µg	0 (0%)	0 (0%)	14 (100%)
Chloramphinicol	30 µg	4 (28.57%)	1 (7.14%)	9(64.28%)
Erythromycin	15 µg	1 (7.14%)	0(0%)	13(92.85%)
Ampicillin	10µg	0 (0%)	1 (7.14%%)	13 (92.85%)
Streptomycin	10 µg	1 (7.14%)	1 (7.14%%)	12(85.71%)
Sulphamethoxazole- trime-thoprim	1.25/23.75 µg	0 (0%)	0 (0%)	14 (100%)

*% Percentage in relation to 14 isolates of *L. monocytogene*

Table (4). Evaluation of the antibiotic resistance, phenotypes and genotypes patterns of *L. monocytogenes* isolates (n=14).

MARPs			(MARG) patterns			
Isolate	**Resistant Antibiotic**	**MARI**	**Macrolides (mefA)**	**β-lactamases ampC**	**Aminogl-ycosides (aad6)**	**MARG**
1.	CTX-DO-CT-E-AMP-SXT-DA	0.7	+	+	-	+
2.	DO-ENR-CT-E-C-SXT-CD	0.7	+	-	-	-
3.	CTX-DO-CT-C-AMP-S-SXT-DA	0.8	-	+	+	+
4.	CTX-ENR-DO-CT-E-C-AMP-S-SXT-DA	1	+	+	+	+
5.	CTX-CT-AMP-SXT-E-DA	0.6	+	+	-	+
6.	CTX-DO-ENR-CT-E-C-AMP-S-SXT-DA	1	+	+	+	+
7.	CTX-DO-ENR-CT-E-C-S-SXT-AA	0.9	+	-	+	+
8.	CTX-DO-CT-E-C-AMP-S-SXT-DA	0.9	+	+	+	+
9.	CTX-DO-CT-C-AMP-E-DA-SXT-S	0.9	+	+	+	+
10.	CTX-DO-CT-E-C-AMP-S-SXT-DA	0.9	+	+	+	+
11.	CTX-CT-AMP-S-SXT-E-D A	0.7	+	+	+	+
12.	CTX-DO-CT-AMP-S-E-SXT-DA	0.8	+	+	+	+
13.	CTX-ENR-DO-CT-E-C-SXT-DA	0.8	+	-	-	-
14.	CTX-ENR-DO-CT-E-C-AMP-S-SXT- DA	1	+	+	+	+

(CTX)Cefotaxime, (DO)Doxycycline, (ENR)Enerofloxacin, (CT)Colistinsulphate,(DA)Clindamycin,(C) Chloramphinicol, (E)Erythromycin, (AMP)Ampicillin, (S) Streptomycin and (SXT) Sulphamethoxazole- trimethoprim
(MAR) index: Multiple antimicrobial resistance index.
(MARPs): The multiple antimicrobial resistance phenotypes of *L. monocytogenes*
(MARG): The multiple antimicrobial resistance genotypes of *L. monocytogenes*

Table (5). The prevalence of detection of antibiotic and arsenic resistance genes in strains isolated from rabbits (n=14)

Resistance gene	Incidence No.	%
*mef*A gene (macrolide resistance)	13/14	92.9%
*amp*C gene (β-lactamase resistance)	11/14	78.5%
*aad*6 gene (aminoglycoside resistance)	10/14	71.4%
*asr*B gene (arsenic resistance)	5/10	35.7%

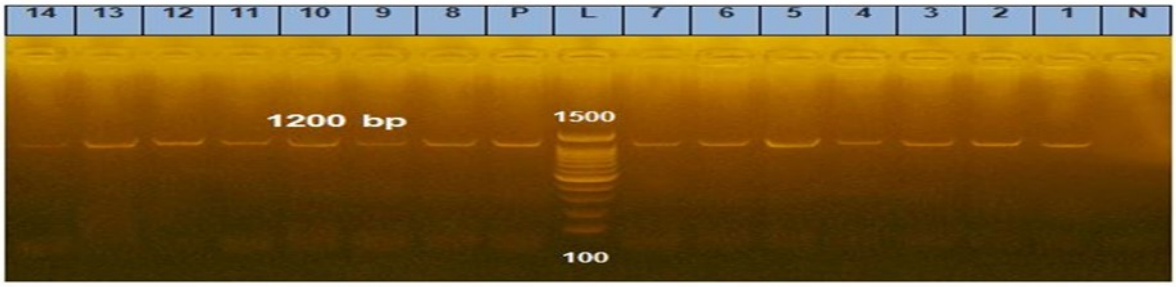

Fig. (1). Agarose gel electrophoresis of PCR for detection of 16S rRNA gene *in L. monocytogenes* at 1200 bp in 14 examined samples. L (Ladder): DNA ladder (100-1500bp). All samples are positive. P: Positive control; N: Negative control.

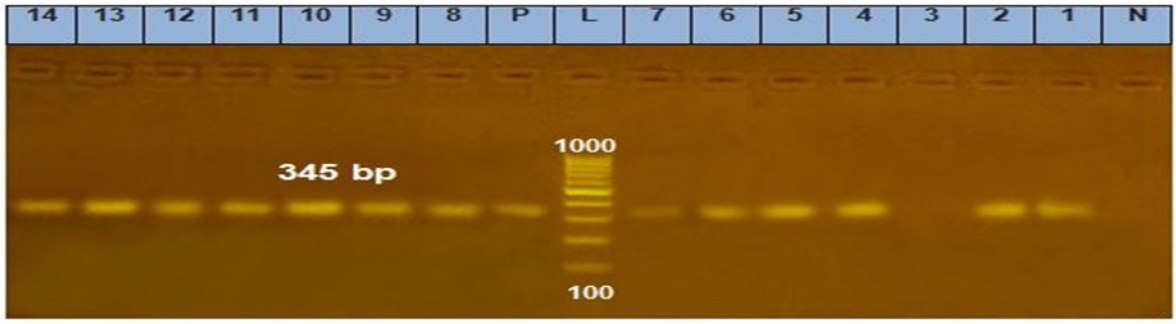

Fig. (2). Agarose gel electrophoresis patern of PCR for detection of *mef*A gene in *L. monocytogenes* at 345 bp in 13/14 examined samples. L (Ladder): DNA ladder (100-1000bp). All samples are positive except one sample lane (3) is negative. P: Positive control; N: Negative control: Negative control.

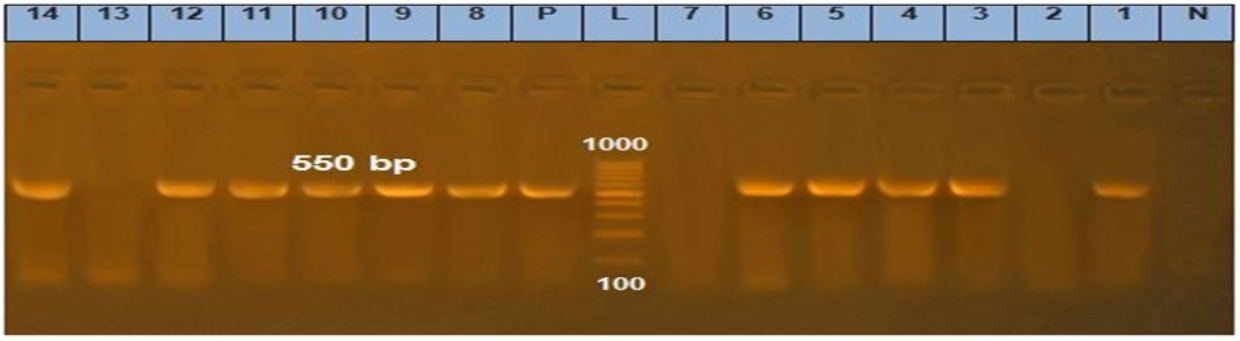

Fig (3). Agarose gel electrophoresis of PCR for detection of *amp*C gene in *L. monocytogenes* at 550 bp in 11/14 examined samples. L (Ladder): DNA ladder (100-1000bp). All samples are positive except samples lanes (2, 7 &13) are negative. P: Positive control; N: Negative control.

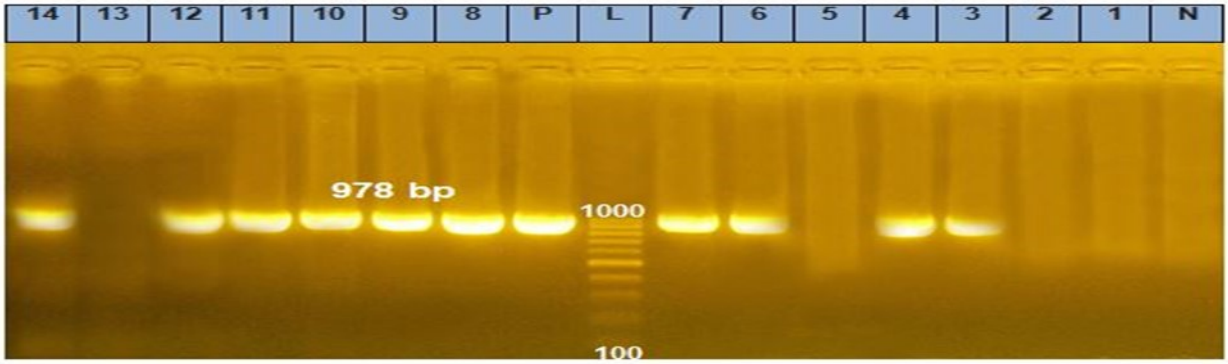

Fig. (4). Agarose gel electrophoresis of PCR for detection of *aad*6 gene in *L.monocytogenes* at 978 bp in 10/14examined samples. L (Ladder): DNA ladder (100-1000bp). All samples are positive except samples lanes (1, 2, 5 &13) are negative. P: Positive control; N: Negative control.

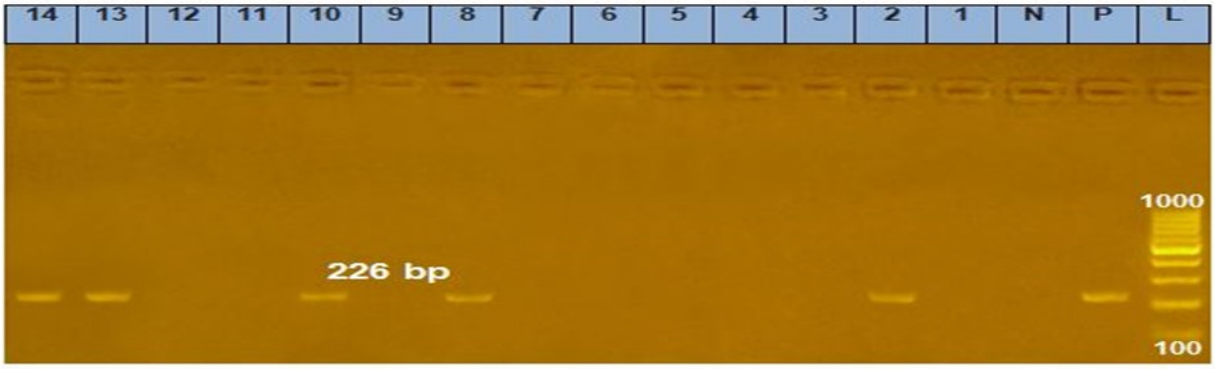

Fig. (5). Agarose gel electrophoresis of PCR for detection of *ars*B gene in *L. monocytogenes* at 226 bp in 5/14 examined samples. L (Ladder): DNA ladder (100-1000bp). Only five samples are positive lanes (2, 8, 10, 13 & 14). P: Positive control; N: Negative control.

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